

FORM PTO-1390

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

10806-106

U.S. APPLIC. NO. (if known, see 37 CFR 1.5)

09/445919

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE98/01368	10 July 1998	11 July 1997

TITLE OF INVENTION

PROSTAGLANDIN DERIVATIVES DEVOID OF SIDE-EFFECTS FOR THE TREATMENT OF GLAUCOMA

APPLICANT(S) FOR DO/EO/US

STJERN SCHANTZ, Johan; RESUL, Bahram; LAKE, Staffan

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US)
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendment has NOT expired.
 - d. have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A **FIRST** preliminary amendment.
 A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. A substitute specification.
15. A change of power of attorney and/or address letter.
16. Other items or information: Copy of published International application, including Search Report.

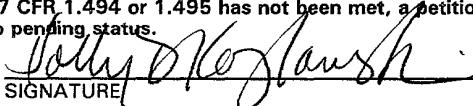
CERTIFICATE OF EXPRESS MAILING

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Date of Deposit: December 14, 1999

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U.S. APPLIC. NO. (if known, see 37 CFR 1.50) 09/445919	INTERNATIONAL APPLICATION NO. PCT/SE98/01368	ATTORNEY'S DOCKET NUMBER 10806-106	
		CALCULATIONS	PTO USE ONLY
17. The following fees are submitted:			
Basic National Fee (37 CFR 1.492(a)(1)-(5)): <input type="checkbox"/> Search Report has been prepared by the EPO or JPO \$840.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00 <input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$760.00 <input checked="" type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$970.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00			
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$ 970.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$ 130.00	
Claims	Number Filed	Number Extra	Rate
Total Claims	20 -20 =	0	x \$18.00 \$
Independent Claims	2 -3 =	0	x \$78.00 \$
Multiple dependent claim(s) (if applicable)		+ \$260.00 \$	
TOTAL OF ABOVE CALCULATIONS =		\$	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).		\$	
SUBTOTAL =		\$	
Processing fee of \$130.00 for furnishing the English translation later than the <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		+ \$	
TOTAL NATIONAL FEE =		\$1100.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property		+ \$	
TOTAL FEES ENCLOSED =		\$1100.00	
		Amount to be: refunded	\$
		charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$1,100.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 04-1133.			
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.			
SEND ALL CORRESPONDENCE TO:  SIGNATURE HOLLY D. KOZLOWSKI NAME 30,468 REGISTRATION NUMBER 14 DECEMBER 1999 DATE			

09/445919

430 Rec'd PCT/PIO 14 DEC 1999

PATENT

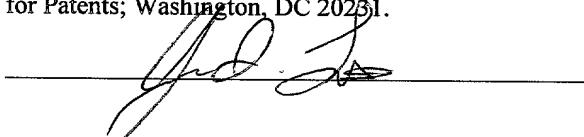
Docket No. 10806-106

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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant: Johan Stjernschantz et al : Paper No.:

Based On: PCT/SE98/01368 : Group Art Unit:

Filing Date: December 14, 1999 : Examiner:

For: **Prostaglandin Derivatives Devoid of Side-Effects for the Treatment of Glaucoma**

PRELIMINARY AMENDMENT

BOX PCT

Assistant Commissioner for Patents

Washington, DC 20231

Dear Sir:

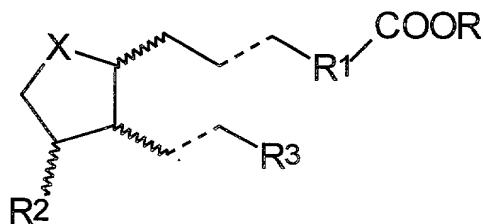
Prior to calculation of the filing fee and first action by the Examiner, please amend the present application as follows:

In the Claims:

Please cancel claim 12.

Please amend claims 3-5 and 8-11 as follows:

3. (Amended) The composition according to claim 1 [or 2], wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, [preferably C₁₋₁₀ alkyl,] cycloalkyl, [preferably C₃₋₈ cycloalkyl,] aryl, arylalkyl, [preferably aryl-C₂₋₅ alkyl,] or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by [a] one or more heteroatoms selected from the group consisting of oxygen, sulfur and nitrogen, cycloalkyl, [preferably C₃₋₇ cycloalkyl,] cycloalkenyl, [preferably C₃₋₇ cycloalkenyl,] aryl or heteroaryl;

X is C-OH or C=O;

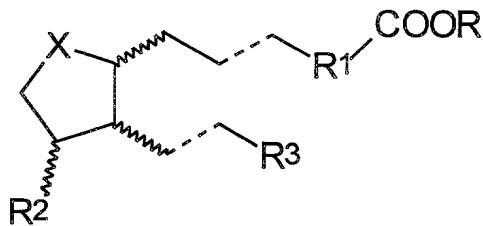
R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, [preferably C₁₋₁₀ alkyl, especially C₁₋₆ alkyl,] or a cycloalkyl[, preferably C₃₋₈ cycloalkyl,] or aryl group; and

R3 is a straight or branched chain saturated or unsaturated alkyl group, [preferably having 3-8 carbon atoms, especially 3-5 carbon atoms,] optionally interrupted by one or more heteroatoms selected from the group consisting of oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C₁₋₅ alkyl, hydroxy and carbonyl groups, [hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure,] and said alkyl group optionally containing a cycloalkyl, [preferably C₃₋₈ cycloalkyl,] aryl or heteroaryl group, [which may be] optionally mono- or independently multi-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

Claim 4, line 1, delete "2 or 3."

Claim 5, line 1, delete "2 or 3."

8. (Amended) The method according to claim 6 [or 7], wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, [preferably C_{1-10} alkyl,] cycloalkyl, [preferably C_{3-8} cycloalkyl,] aryl, arylalkyl, [preferably aryl- C_{2-5} alkyl,] or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by [a] one or more heteroatoms selected from the group consisting of oxygen, sulfur and nitrogen, cycloalkyl, [preferably C_{3-7} cycloalkyl,] cycloalkenyl, [preferably C_{3-7} cycloalkenyl,] aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, [preferably C_{1-10} alkyl, especially C_{1-6} alkyl,] or a cycloalkyl[, preferably C_{3-8} cycloalkyl,] or aryl group; and

R3 is a straight or branched chain saturated or unsaturated alkyl group, [preferably having 3-8 carbon atoms, especially 3-5 carbon atoms,] optionally interrupted by one or more heteroatoms selected from the group consisting of oxygen, sulfur and nitrogen, each carbon

atom optionally being substituted with a substituent selected from C₁₋₅ alkyl, hydroxy and carbonyl groups, [hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure,] and said alkyl group optionally containing a cycloalkyl, [preferably C₃₋₈ cycloalkyl,] aryl or heteroaryl group, [which may be] optionally mono-or independently multi-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

9. (Amended) The [composition] method according to claim 6, [7 or 8,] wherein the prostaglandin analogue is 15(R,S)-16,16-trimethylene-PGE₂ or an alkyl ester thereof.

10. (Amended) The [composition] method according to claim 6, [7 or 8,] wherein the prostaglandin analogue is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE₂ or an alkyl ester thereof.

Claim 11, line 1, replace "any one of claims 6-10" with --claim 6--.

Please add the following claims 13-21:

--13. (NEW) The composition according to claim 3, wherein R is C₁₋₁₀ alkyl, C₃₋₈ cycloalkyl or aryl-C₂₋₅ alkyl.--

--14. (NEW) The composition according to claim 3, wherein R1 is C₃₋₇ cycloalkyl or C₃₋₇ cycloalkenyl.--

--15. (NEW) The composition according to claim 3, wherein R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, wherein R4 is C₁₋₁₀ alkyl or C₃₋₈ cycloalkyl.--

--16. (NEW) The composition according to claim 3, wherein R3 is a straight or branched chain saturated or unsaturated alkyl group having 3-8 carbon atoms, optionally interrupted by one or more heteroatoms selected from the group consisting of oxygen, sulfur and nitrogen, each carbon atom optionally substituted with a substituent selected from C₁₋₅ alky, hydroxy and carbonyl groups, wherein the hydroxy and carbonyl are attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a C₃₋₈ cycloalkyl, optionally mono- or independently tri-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen.--

--17. (NEW) The composition according to claim 3, wherein R4 is C₁₋₆ alkyl and R3 is a straight or branched chain saturated or unsaturated alkyl group having 3-5 carbon atoms.--

--18. (NEW) The method according to claim 6, wherein R is C₁₋₁₀ alkyl, C₃₋₈ cycloalkyl or aryl-C₂₋₅ alkyl.--

--19. (NEW) The method according to claim 6, wherein R1 is C₃₋₇ cycloalkyl or C₃₋₇ cycloalkenyl.--

--20. (NEW) The method according to claim 6, wherein R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, wherein R4 is C₁₋₁₀ alkyl or C₃₋₈ cycloalkyl.--

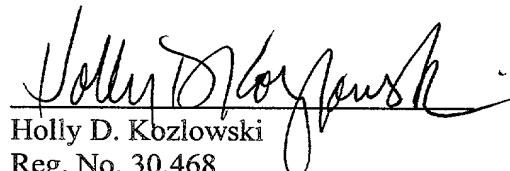
--21. (NEW) The method according to claim 6, wherein R3 is a straight or branched chain saturated or unsaturated alkyl group having 3-8 carbon atoms, optionally interrupted by

one or more heteroatoms selected from the group consisting of oxygen, sulfur and nitrogen, each carbon atom optionally substituted with a substituent selected from C₁₋₅ alky, hydroxy and carbonyl groups, wherein the hydroxy and carbonyl are attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a C₃₋₈ cycloalkyl, optionally mono- or independently tri-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen.--

REMARKS

The claims are amended herein to omit their multiple dependency and for various matters of form in accordance with customary U.S. patent practice. Support for claims 13-21 may be found in original claims 3 and 6. Since these changes do not involve any introduction of new matter, entry is believed to be in order and is respectfully requested.

Respectfully submitted,


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512432.01

PROSTAGLANDIN DERIVATIVES DEVOID OF SIDE-EFFECTS FOR THE TREATMENT OF GLAUCOMA

The invention is related to a method of treating glaucoma and ocular hypertension utilizing prostaglandin analogues or derivatives that are devoid of, or have reduced melanogenic effect in the eye. The invention also relates to ophthalmic compositions containing prostaglandin compounds devoid of, or have reduced melanogenic effect in the eye.

Glaucoma is an eye disorder characterized by increased intraocular pressure, excavation of the optic nerve head, and gradual loss of the visual field. An abnormally high intraocular pressure is commonly known to be detrimental to the eye, and there are clear indications that in glaucoma the intraocular pressure is the most important factor causing degenerative changes in the retina and the optic nerve head. The exact pathophysiological mechanism of open angle glaucoma is, however, still unknown. Unless treated, glaucoma may lead to blindness, the course of the disease typically being slow with progressive loss of vision.

The intraocular pressure (IOP) can be defined according to the formula (1):

$$(1) \quad IOP = Pe + (Ft - Fu) \times R$$

where Pe is the episcleral venous pressure, Ft the formation of aqueous humor, Fu the part of the aqueous humor which exits the eye through the uveoscleral outflow pathway, and R is the resistance in the trabecular outflow pathway. The aqueous humor in the anterior and posterior chambers of the eye is produced by the ciliary processes behind the iris. It then flows through the pupil into the anterior chamber, and normally exits the eye through the trabecular meshwork and Schlemm's canal into the episcleral veins outside the eye globe. However, part of the aqueous humor may leave the eye through the uveoscleral outflow route. The flow in this route is regarded as only minimally influenced by the intraocular pressure (Bill, 1975).

The intraocular pressure in humans is normally in the range of 12-22 mmHg. At higher pressures, e.g. above 22 mmHg, there is an increased risk that the eye may be

damaged. In one particular form of glaucoma, normal tension glaucoma, damage may, however, occur at intraocular pressure levels that are within the normal physiological range. The opposite situation is also known, i.e. some individuals may exhibit an abnormally high intraocular pressure without any manifest defects in the visual field or the optic nerve head. Such conditions usually are referred to as ocular hypertension.

Glaucoma treatment can be given by means of drugs, laser or surgery. In drug treatment the purpose is to reduce either the formation of aqueous humor (F_t), or the resistance to outflow of aqueous humor (R), which according to formula (1) above will result in reduced intraocular pressure; alternatively to increase the outflow of aqueous humor through the uveoscleral route which according to formula (1) also reduces the intraocular pressure.

Prostaglandins and typically PGF_{2α}, its esters and analogues, reduce the intraocular pressure mainly by increasing the uveoscleral outflow of aqueous humor (Crawford and Kaufman, 1987; Nilsson et al., 1989; Toris et al., 1993; Stjernschantz et al., 1995). The use of prostaglandins and their derivatives is described in several patents and patent applications, for instance in US 4,599,353 (Bito), US 4,952,581 (Bito), WO89/03384 (Resul and Stjernschantz), EP 170258 (Cooper), EP 253094 (Goh), and in EP 308135 (Ueno).

Prostaglandins are fatty acids usually derived from the precursors eicosatrienoic, eicosatetraenoic and eicosapentaneoic acid through metabolic steps involving oxygenation. Naturally occurring prostaglandins typically have the general structure shown in Fig. 1.

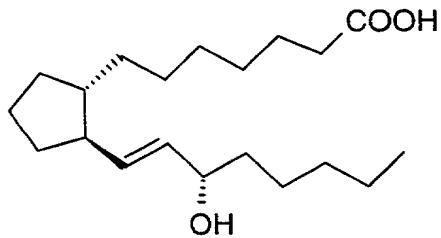


Fig. 1

The prostaglandins accordingly carry a cyclopentane ring to which two carbon chains link, the upper chain usually being called the alpha chain and the lower chain usually being called the omega chain. The prostaglandins are classified in subgroups A, B, C, D, E, F, G, H, I and J depending on the structure and substituents in the cyclopentane ring as shown in Fig. 2.

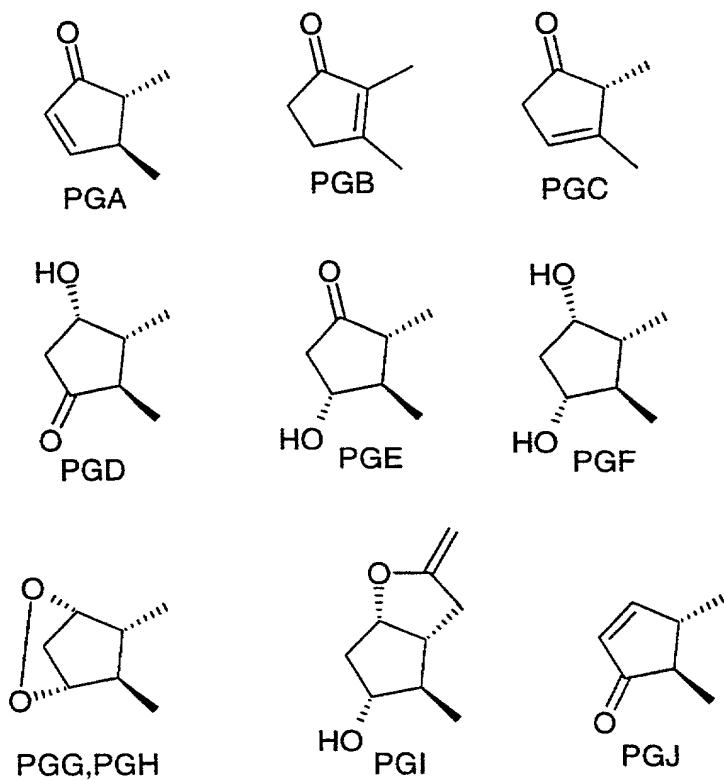


Fig. 2

The alpha chain is a 7 carbon carboxy-terminated aliphatic chain whereas the omega chain is an 8 carbon methyl-terminated aliphatic chain. Depending on the number of double bonds in these chains subscripts of 1 to 3 are given. In prostaglandins with subscript 1, e.g. PGF_{1 α} , the double bond is situated between carbons 13 and 14 in the omega chain, and it exhibits trans configuration in naturally occurring prostaglandins. In prostaglandins with subscript 2, e.g. PGF_{2 α} , an additional double bond in the cis configuration is situated between carbons 5 and 6 in the alpha chain, and finally in

prostaglandins with subscript 3, a third double bond is situated between carbons 17 and 18 in the omega chain. This double bond also exhibits cis configuration in naturally occurring prostaglandins. All naturally occurring prostaglandins carry a hydroxyl group on carbon 15, which is essential for biological activity.

The receptor system for the naturally occurring prostaglandins has only recently been elucidated. Thus most of the prostaglandin receptors have been pharmacologically characterized and their molecular structure identified by molecular biological techniques (Coleman et al., 1994). There are specific receptors for the naturally occurring prostaglandins. The receptors for PGD, PGE, PGF, PGI₂ (prostacyclin) and TXA₂ (thromboxane) being abbreviated DP, EP, FP, IP, and TP, respectively. Furthermore it has been shown that the EP receptor can be subdivided into four receptors, namely the EP₁, EP₂, EP₃, and EP₄ receptors. Specific tissues or cells may express only a few or many of these receptors, depending on the evolutionary development of this autacoid system in different species. Thus for instance it has been shown that the cat iris sphincter muscle expresses predominantly FP receptors that are functionally coupled and mediate constriction of the pupil, while the corresponding smooth muscle of the bovine eye expresses EP₁ and TP receptors, activation of either of which will result in contraction of the muscle. A compound which binds to a specific receptor and activates it is called an agonist while a compound that only binds to a receptor without activating it is called an antagonist.

With respect to the practical usefulness of many of the prostaglandins and their derivatives as suitable drugs for treatment of glaucoma or ocular hypertension, a limiting factor may be their property of causing increased pigmentation of the iris in the eye (Stjernschantz and Alm, 1996). Thus, the colour of the iris during chronic treatment in monkeys and in man tends to become darker, turning into brown. While this apparently has no negative medical consequences, it is a clear disadvantage from a cosmetic point of view, particularly in patients undergoing treatment only in one eye. It would thus be desirable to identify prostaglandins which effectively reduce the intraocular pressure without causing the side-effect of increased iridial pigmentation.

We have now unexpectedly found that prostaglandin derivatives and analogues which are selective agonists for the EP₁ subgroup of prostanoid receptors fulfil the criteria for a prostaglandin analogue which effectively reduces the intraocular pressure without causing increased production of pigment (melanogenesis) in the iris. The background of this finding is that in a study to identify the prostanoid receptor subtypes in the human iridial melanocytes we have found that these cells express in their cell membranes the FP, EP₂, and EP₃ receptors, but not the EP₁ and TP receptors. Furthermore, we have investigated the intraocular pressure reducing effect of several relatively selective EP₁ agonists and found that these prostaglandin analogues effectively and potently reduce the intraocular pressure both in cats and monkeys.

Therefore, it is now apparent that, by using selective EP₁ receptor agonists, the intraocular pressure can be reduced in primates, and thus also in man, without increased, or with significantly reduced melanogenesis as side-effect, since the melanin producing cells, the melanocytes, lack the specific receptor necessary for the transmembrane signalling into the cell. While we at present have no clinical evidence that such selective EP₁ agonists do not cause increased pigmentation of the iris, since the induction time for this phenomenon to occur often is 6-12 months, and thus extremely long and costly experiments have to be carried out in primates, we can nevertheless conclude from relevant *in vitro* studies that such increased pigmentation would not occur in the absence of the specific signalling receptor in the cell membrane of the melanocytes.

Accordingly, high selectivity or specificity to the EP₁ receptor compared to other prostaglandin receptors in the eye characterizes the compounds to be used in the method or compositions according to the present invention. It need not to be said that the more selective the compound is for the EP₁ receptor, the better results are obtained but a certain advantage is of course achieved also in cases of some interaction with other receptors. High selectivity in this connection means that the effect on the EP₁ receptor is at least more than 5 times, especially more than 10 times, and in particular more than 100 or 1000 times the effect on the other prostaglandin receptors.

The specific prostaglandin analogues that we have used for exemplifying and proving this invention were PGF_{2 β} (1), PGF_{2 β} isopropyl ester (2), 17-phenyl-18,19,20-trinor-PGE₂ (3), 17-phenyl-18,19,20-trinor-PGE₂ isopropyl ester (4), 15(R,S)-16,16-trimethylene-PGE₂ (5), 15(R,S)-16,16-trimethylene-PGE₂ methyl ester (6), and 13,14-dihydro-17-(3-fluorophenyl)-18,19,20 trinor-PGE₂-isopropyl ester (7). All these analogues are relatively selective EP₁ receptor agonists. The receptor profiles of the test compounds are presented in Table I.

Table I. Receptor profile of prostaglandin analogues tested (EC-50 values expressed as moles/l in functional receptor assays).

Prostaglandin	FP	EP ₁	EP ₂	EP ₃	DP/IP	TP
1	5x10 ⁻⁶	10 ⁻⁶	10 ⁻⁵	>10 ⁻⁴	>10 ⁻³	
	>10 ⁻³					
3	10 ⁻⁷	2x10 ⁻⁸	>10 ⁻⁴		>10 ⁻⁴	>10 ⁻⁴
	>10 ⁻⁴					
5	2x10 ⁻⁵	6x10 ⁻⁹	2x10 ⁻⁷	3x10 ⁻⁸ #	>10 ⁻⁴	>10 ⁻⁴
7	6x10 ⁻⁷	4x10 ⁻⁸	5x10 ⁻⁵	10 ⁻⁶ #	>10 ⁻⁴	>10 ⁻⁴

estimated based on difference in receptor assay between guinea pig vas deferens and chick ileum.

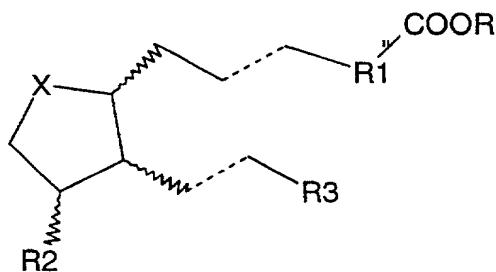
In one aspect, the invention relates to the use of selective prostaglandin EP₁ receptor agonists devoid of melanogenic effect for the treatment of glaucoma or ocular hypertension. The method for treating glaucoma or ocular hypertension comprises contacting the surface of the eye with an effective intraocular pressure reducing amount of a composition, containing an EP₁ selective prostaglandin as aforesaid, in order to reduce the intraocular pressure and to maintain said pressure at a reduced level. The composition usually contains about 0.1-100 μ g, especially 1-30 μ g per application of the active substance. The composition is applied topically on the eye 1-3 times daily.

The prostaglandin derivative is mixed with an ophthalmologically compatible vehicle known per se. The vehicle which may be employed for preparing compositions of this invention comprises aqueous solutions, e.g. physiological saline, oil solutions, or ointments. The vehicle may furthermore contain ophthalmologically compatible preservatives such as e.g. benzalkonium chloride, surfactants, such as polysorbate 80, liposomes or polymers, for example methyl cellulose, polyvinyl alcohol, polyvinyl pyrrolidone and hyaluronic acid; these may be used for increasing the viscosity. Furthermore it is also possible to use soluble or insoluble drug inserts.

In another aspect, the invention relates to ophthalmological compositions for medical treatment of glaucoma or ocular hypertension which comprise an effective intraocular pressure reducing amount of a prostaglandin analogue which is a selective agonist of EP₁ receptors as defined above and an ophthalmologically compatible carrier. The effective amount usually comprises a dose of about 0.1-100 µg in about 10-50 µl of the composition. The compositions according to the present invention are clear improvements over the prior art prostaglandin compositions due to the selectivity of the active compound for EP₁ receptors compared to other prostaglandin receptors with the risk for pigmentation eliminated or at least substantially reduced.

In still another aspect, the invention relates to the use of the prostaglandin analogue for the preparation of a medicament for treatment of glaucoma and ocular hypertension.

Preferably, the prostaglandin analogue is derived from PGF or PGE type prostaglandins. Particularly, the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably C₁₋₁₀ alkyl, cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl, arylalkyl, preferably aryl-C₂₋₅ alkyl, or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably C₃₋₇ cycloalkyl, cycloalkenyl, preferably C₃₋₇ cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, preferably C₁₋₁₀ alkyl, especially C₁₋₆ alkyl, or a cycloalkyl, preferably C₃₋₈ cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C₁₋₅ alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

Aryl is preferably substituted or unsubstituted phenyl.

Exemplary heteroaryl groups are thiophene, furan, thiazole, isothiazole, oxazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine.

Aryl, heteroaryl and cycloalkyl may be mono- or independently di- or multi-substituted by C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen.

Unsaturated alkyl may contain one or more double and/or triple bonds.

Halogen is fluorine, chlorine, bromine or iodine, especially fluorine, chlorine or bromine.

The prostaglandins may be in epimeric mixtures as well as in the form of the individual epimers.

The invention is illustrated by means of the following non-limiting examples:

Identification of prostaglandin receptors. The prostaglandin receptors were identified using the reversed transcriptase polymerase chain reaction (RT-PCR) technique. Specific primers were designed for the FP, EP₁, EP₂, EP₃, and TP receptors. The primers used in the assays are presented in Table II. RT-PCR was performed on mRNA isolated from human iridial melanocytes in culture. The cultured cells were used for preparing mRNA. The RT-PCR mix was analysed on agarose gel and bands of expected size were cloned and sequenced. The deduced sequences were analysed for similarity to each prostaglandin receptor sequence.

Methods. Human iridial melanocytes were isolated and cultured according to Hu et al. (1993) and used in early passages. Cells were grown to confluence and harvested for the mRNA enrichment.

mRNA was isolated using Dynal's mRNA Direct System (Dynal A/S, Norway) according to the manufacturer's instructions. 100.000-200.000 human melanocyte cells were used in the enrichment. mRNA is covalently bound to an oligo-dT labeled Dynabead. Using reverse transcriptase the first strand cDNA is synthesized directly on

the Dynabeads with the oligo-dT as a reverse transcriptase primer. The second strand cDNA is synthesized using a known 3' sequence primer from respective prostaglandin receptor, resulting in double stranded cDNA. The same set of Dynabeads was used for each receptor RT-PCR. Receptor specific primers were used for PCR amplifying DNA from Dynabead bound cDNA according to the manufacturer's instructions. For the FP and EP₃ receptor reactions the PCR was performed in 50 µl final volume with 5% DMSO, 200 µM dNTPs and 20 pmoles of each primer. For the other receptors hot start with AmpliWax pellets (Perkin Elmer, USA) was used in a final volume of 75 µl with 5% DMSO, 200 µM dNTPs and 20 pmoles of each primer.

Table II. Prostaglandin receptor specific primers.

FP primers:

Primary primers; CAC AAC CTG CCA GAC GGA AAA C and CCA GTC TTT
GAT GTC TTC TGT G

Secondary primers; CAG TAA TCT TCA TGA CAG TGG G and TTG TAG AAA
CAC CAG GTC CTG G

EP₁ primers:

primary primers; TGT GGC ATG GCC GTG GAG and ACC AAC ACC AGC ATT
GGG C

secondary primers; CTG CAG GGA GGT AGA GCT C and GGC ACG TGG TGC
TTC ATC G

EP₂ primers:

primary primers; CAA CCA TGC CTA TTT CTA CAG C and TCT CGC TCC AAA
CTT GGC TG

secondary primers; CTA CGT GGA CAA GCG ATT GGC and TGG TTG ACG
AAC ACT CGC AC

EP₃ primers:

primary primers; GGG ATC CAA GAT CTG GTT CAG and GCC TTC CCG ATC
ACC ATG CTG

secondary primers; CGC AAG AAG TCG TTC CTG CTG and CAC CAA GTC
CCG GGC CAC TG

TP primers:

primary primers; CTG GTG ACC GGT ACC ATC GTG GTG T and GTA GAT CTA
CTG CAG CCC GGA GCG C

secondary primers; TCG CTA CAC CGT GCA ATA CC and GGC TGG AGG GAC
AGC GAC

The PCR mix from these reactions was analyzed on a 1% LMP agarose gel (BioRad Laboratories, USA). DNA fragments of the expected size were TA-cloned using a TA-cloning kit according to the manufacturer's instructions (Invitrogen Inc., USA), and sequenced on an Applied Biosystem Model 373A DNA sequencing system (Applied Biosystems Inc., USA) according to Applied Biosystems' protocol for their Taq Dye Dideoxy Terminator cycle sequencing kit. The generated primary data were processed on a VAX computer using the sequence analysis programs from Genetics Computer Group Inc., Madison, USA (Devereux, J., et al., Nucleic Acids Research 12 (1): 387-395 (1984)).

Results: In human iridial melanocytes based on our RT-PCR we could show an expression of FP, EP₂ and EP₃ receptors. However, we were not able to show the presence of the EP₁ and TP receptors (Table III). As positive controls we amplified the expected EP₁ and TP fragments with the same primers from a human kidney

cDNA library. We enriched poly A mRNA from human iridial melanocytes isolated at two different times and performed the PCR reactions several times with identical result.

Table III. RT-PCR (secondary PCR primers) of human iridial melanocyte mRNA using prostaglandin receptor specific primers (see Table II).

Gene	Correct fragment size (bp)		Sequence analysis
	<u>Observed</u>	<u>Expected</u>	
FP	+	489	Identity with FP
EP ₁	-	397	-
EP ₂	+	501	Identity with EP ₂
EP ₃	+	372	Identity with EP ₃
TP	-	484	-

SYNTHESIS OF PROSTAGLANDIN DERIVATIVES

The structures of the end compounds prepared in the Examples are shown in Scheme 1 provided at the end of the description.

Example 1: PGF_{2 β} (compound 1)

The title compound was purchased from Cayman Chemicals Company, Ann Arbor Michigan, USA.

Example 2: PGF_{2 β} isopropyl ester (compound 2)

DBU (163.5 mg, 1.01 mmol) was added to a stirred solution of PGF_{2 β} (Cayman Chemicals) (60 mg, 0.169 mmol) in acetone (20 ml) at 0 °C. The mixture was allowed

to warm to room temperature, when isopropyl iodide (222.6 mg, 1.34 mmol) was added dropwise. After 48 h (TLC monitoring), the mixture was diluted with ethyl acetate (40 ml), washed with brine (30 ml), citric acid 3% (2x40 ml) and sodium hydrogen carbonate 5% (2x30 ml) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel using ethyl acetate : acetone 3:1 as eluent. This afforded a colorless oil, yield 46 mg (68%). ^1H NMR (CDCl_3) d 1.3 (d, 6H), 1.6-1.7 (dm, 4H), 2.0-2.2 (dm, 6H), 2.3 (t, 2H), 4.0-4.1 (m, 3H), 5.0 (sept, 1H), 5.5 (m, 2H), 5.6 (m, 2H). ^{13}C NMR (CDCl_3) d 135.9, 132.2, 130.5, 128.0, 75.3, 74.8, 72.85, 67.6, 56.23, 52.25, 51.59, 42.32, 37.35, 33.44, 31.74, 29.14, 26.66, 24.79, 22.6, 21.8, 14.03.

Example 3: 17-Phenyl-18,19,20-trinor-PGE₂ (compound 3)

The title compound was purchased from Cayman Chemicals Company, Ann Arbor Michigan, USA.

Example 4: 17-Phenyl-18,19,20-trinor-PGE₂ isopropyl ester (compound 4)

DBU (43.5 mg, 0.29 mmol) in acetonitrile (1 ml) was added dropwise to a stirred solution of compound 3 (22.1 mg, 0.057 mmol) in acetonitrile (3 ml) at 0 °C. The mixture was allowed to warm to room temperature whereupon isopropyl iodide (78.0 mg, 0.46 mmol) in acetonitrile (2 ml) was added dropwise. After being stirred for 12 h (TLC monitoring), the reaction mixture was quenched with water (8 ml), the mixture was extracted with ethyl acetate (2x50ml), and the extract was washed with brine (10 ml), citric acid 3% (10 ml), and finally sodium hydrogen carbonate 5% (10 ml). After drying with anhydrous sodium sulfate, the solvent was removed in vacuo and the residual oil was chromatographed on silica gel using ethyl acetate as eluent. This afforded 230 mg of the product (69%) of the title compound as a colorless oil: R_f = 0.516 (ethyl acetate : acetone : HOAc 1:1:0.02); ^1H NMR (CDCl_3) d 0.89 (m, 3H), 1.3 (d, 6H), 2.6-2.8 (m, 2H), 4.1 (m, 2H), 5.0 (m, 1H), 5.3-5.7 (dm, 4H), 7.2 (m, 5H). ^{13}C NMR (CDCl_3) d 10.9, 13.9, 21.8, 22.9, 23.8, 24.49, 24.8, 25.17, 25.6, 26.68, 28.93, 30.45, 31.77, 33.90, 34.01, 34.07, 38.8, 46.22, 53.3, 54.48, 66.83, 67.62, 68.18, 71.77, 72.21, 76.35, 77.00, 77.2,

77.64, 125.93, 126.46, 128.39, 128.44, 128.79, 130.63, 130.81, 131.04, 137.79, 213.88.

Example 5: 15RS-16,16-trimethylene-PGE₂ (compound 5)

To a stirred solution of 15RS-16,16-trimethylene-PGE₂ methyl ester (52 mg, 0.13 mmol) in acetone (0.4 ml) and phosphate buffer pH 7 (4 ml) was added lipase VII (40 mg). The mixture was stirred at room temperature for 24 h (TLC monitoring). The mixture was quenched with ethanol (3 ml) and extracted with ethyl acetate (2x10 ml). The organic layer was washed with brine, dried (sodium sulfate), and concentrated in vacuo furnishing 46 mg of the product as an oil.

Example 6: 15RS-16,16-trimethylene-PGE₂ methyl ester (compound 6)

The synthesis of 15RS-16,16-trimethylene prostaglandin E₂ (Skotnicki, S. et al. 1977) is schematically shown in Scheme 2. Bold figures in the following refer to respective structures in Scheme 2.

Ethyl 2,2-trimethylenehexanoate (9)

To a stirred solution of N-isopropylcyclohexylamine (56.2 g, 398 mmol) in THF (400 ml) at -78 °C was added rapidly n-BuLi (159 ml, 398 mmol of 2.5 M in hexane). To the resulting solution was added dropwise ethyl cyclobutanecarboxylate (**8**) (50 g, 390 mmol) and stirred for 30 min, then warmed to 0 °C and dropped into a solution of n-butyl iodide (159 ml, 398 mmol of 2.5 mol in hexane) in DMSO (200 ml). The reaction mixture was stirred for 1 h at room temperature (TLC monitoring). The salt was removed by filtration and the filtrate was concentrated in vacuum. The residue was dissolved in hexane and washed with HCl 2%, brine and water, then dried over sodium sulfate, and evaporated in vacuo. The residual oil was distilled (49-56 °C, 1 mmHg) to give 26.5 g (37%) of the product.

¹H NMR (CDCl₃) δ 0.9 (t, 3H), 1.2 (t, 3H), 1.8-2.0 (dm, 5H), 2.2-2.5 (m, 3H), 4.2 (m, 2H).

2,2-Trimethylenehexan-1-ol (10)

To a stirred solution of ethyl 2,2-trimethylenehexanoate (**9**) (26.5 g, 144 mmol) in dry toluene (100 ml) was added dropwise DIBAL-H (206 ml, 289 mmol of 1.4 mol in toluene) at 0 °C. The resulting solution was stirred at room temperature for 3 h (TLC monitoring), and then poured into iced HCl 5%. The organic layer was separated and washed with HCl 5%, brine, dried, filtered and concentrated to give 30 g of the product as an oil. ^1H NMR (CDCl_3) δ 0.9 (t, 3H), 1.8-2.0 (dm, 5H), 2.5 (m, 1H), 3.0 (m, 1H), 3.6 (m, 2H).

2,2-Trimethylenehexaldehyde (**11**)

To a solution of 2,2-trimethylenehexan-1-ol (**10**) (30 g, 210 mmol), in DME (400 ml), was added dicyclohexanecarbodiimide (DCC) (130 g, 630 mmol), DMSO (120 ml) and orthophosphoric acid (10.3 g). The mixture was stirred at room temperature for 3 h (TLC monitoring), and filtered. The filtrate was diluted with dichloromethane (300 ml), and washed with water. The organic layer was separated. The residue was removed by filtration. The filtrate was washed with brine (100 ml), dried and concentrated in vacuum. The residue was purified by column chromatography on silica gel using hexane as eluent to give the title product (17.3 g) as an oil.

^1H NMR (CDCl_3) δ 0.9 (t, 3H), 1.2 (t, 3H), 1.8-2.0 (dm, 5H), 8.8 (s, 1H).

4,4-Trimethylene-1-octyn-3-ol (**12**)

To a solution of lithium acetylide-ethylenediamine complex (12.2 g, 132 mmol) in DMSO (10 ml) was added a solution of 2,2-trimethylenehexaldehyde (**11**) (17 g, 120 mmol) in DMSO (20 ml) at 0 °C under N_2 . The mixture was stirred at room temperature for 24 h (TLC monitoring) and then poured into an ice-cold HCl 2% (50 ml) and ether (50 ml). The organic layer was separated and the aqueous layer was extracted with ether (50 ml), the combined organic phases were washed with brine, dried, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate 5:1 as eluent, which gave **12** (7.6 g, 38%) as an oil.

E-Tributyltin-4,4-trimethylene-1-octene-3-ol (**13**)

A mixture of 4,4-trimethylene-1-octyn-3-ol (**12**) (5.0 g, 30 mmol), tributyltin hydride (14.6 ml, 54.2 mmol), and AIBN (30 mg) was stirred at 130 °C for 24 h (TLC monitoring). The residue was chromatographed on silicagel using hexane and hexane:ether 9:1, respectively, as eluent, to give the title compound (**13**) (12.54 g, 91.4%) as an oil.

E-Tributyltin-4,4-trimethylene-3-trimethylsilyloxy-1-octene (**14**)

To the mixture of E-tributyltin-4,4-trimethylene-1-octene-3-ol (**13**) (7 g, 15.3 mmol) in DMF (100 ml) was added imidazole (2.1 g, 30.6 mmol) and trimethylsilyl chloride (2.5 g, 23.0 mmol). The reaction mixture was stirred at room temperature for 1 h (TLC monitoring). The mixture was partitioned between water (200 ml) and ether (200 ml). The organic phase was dried and evaporated in vacuo. The residue was chromatographed on silica gel using hexane as eluent to give **14** (5.53 g).

11,15-bis Trimethylsilyloxy-16,16-trimethylene-5,6-didehydro-PGE₂ methyl ester (**17**)

A dry 100-ml three-necked flask was charged with copper(I)cyanide (928 mg, 10.4 mmol) and a magnetic bar. The flask was capped with a rubber septum and heated under vacuum to remove any trace of water, and cooled to 0 °C under N₂. Dry THF was added and followed by methyl lithium (14 ml, 22.4 mmol of 1.6 mol in diethyl ether), via a syringe. The mixture was stirred at 0 °C for 15 min. during which the suspension became clear and homogeneous. A solution of E-tributyltin-4,4-trimethylene-3-trimethylsilyloxy-1-octene (**14**) (5.9 g, 11.2 mmol) in THF (10 ml) was added, via a syringe, at 0 °C and stirred at room temperature for 30 min. To the resulting solution a solution of 4-(t-butyldimethylsilyloxy)-cyclopentenone (**15**) (1.7 g, 8 mmol) in THF (6 ml), trimethylsilyl chloride (4.35 g, 40 mmol) and triethylamine (8.1 g, 80 mmol) was added, at -70 °C, successively and stirred at -70 °C for another 15 min, then for 15 min at 0 °C. The mixture was partitioned between hexane (600 ml) and water (300 ml). The organic layer was separated, dried over sodium sulfate, filtered and concentrated in vacuo to give the crude silyl enol ether as a clear oil. To the stirred solution of the silyl enol ether in THF (50 ml), under N₂, at -30 °C methyl lithium (7.7 ml, 12.3 mmol of 1.6 mol in diethyl ether) was added and stirred for 30 min followed by addition of a freshly prepared methyl-1-triflate-2-hexynoate (**16**)

(Erhardt, P.W., et al. 1987; Caldwell A. G., et al. 1979) and stirred at -40 °C for 5 min. The resulting solution was quenched with saturated aqueous ammonium chloride solution (30 ml) and extracted with ether (3x 100 ml), dried on sodium sulfate, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate (1:1) as eluent, to give a clear oil of a mixture of 15RS isomers (2.71 g, 57.3%) R_f = 0.36 (SiO₂, ether : hexane 1:1). ¹H NMR (CDCl₃) δ 0.2 (dm, 12H), 0.8-0.9 (ms, 18H), 1.8 (m, 2H), 2.3 (m, 4H), 3.7 (s, 3H), 3.9-4.1 (dm, 2H), 5.5-5.6 (2H). The ¹H NMR was also performed on the desilylated analogue, 16,16-trimethylene-5,6-didehydro-PGE₂ methyl ester. ¹H NMR (CDCl₃) δ 0.9 (t, 3H), 1.2-1.3 (m, 3H), 1.9-2.1 (m, 4H), 3.7 (s, 3H), 4.1 (m, 2H), 5.6-5.9 (dm, 2H).

11,15-bis Trimethylsilyloxy-16,16-trimethylene-PGE₂ methyl ester

To a stirred solution of 11,15-bis trimethylsilyloxy-16,16-trimethylene-5,6-didehydro-PGE₂ methyl ester (**17**) (500 mg, 0.8 mmol), in benzene : cyclohexane 1 : 1 (50 ml) was added Pd-BaSO₄ (250 mg) and quinoline (250 mg) and stirred at -40 °C under H₂ atmosphere for 5 h (TLC monitoring). The reaction mixture was diluted with ether and filtered through celite, and concentrated in vacuum. The residue was chromatographed on silica gel using hexane : ethyl acetate 9:1 to give 442 mg of the corresponding product.

16,16-Trimethylene-PGE₂ methyl ester (**6**)

To the solution of 11,15-bis trimethylsilyloxy-16,16-trimethylene-PGE₂ methyl ester (374 mg, 0.589 mmol) in THF (18 ml) was added HF 40% (3.5 ml) in THF (1 ml) at 0 °C. The reaction mixture was stirred for 5 h (TLC monitoring) and then poured into a mixture of sodium hydrogen carbonate 5% (30 ml) and ethyl acetate (50 ml). The organic layer was separated and the aqueous layer was washed with ethyl acetate (2x30 ml). The organic layers were pooled and dried, on sodium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate 1:1, and ethyl acetate successively to give **6** (75 mg, 31%), as an oil.

¹H NMR (CDCl₃) δ 0.9 (t, 3H), 1.3 (t, 6H), 2.0-2.6 (mm, 9H), (dm, 5H), 3.6 (s, 3H), 4.1 (m, 2H), 5.4 (m, 2H), 5.6-5.8 (dm, 2H); ¹³C NMR (CDCl₃) δ 14.222, 14.9, 23.7,

24.7, 25.2, 26.2, 26.5, 26.6, 26.8, 29.7, 33.4, 36.5, 44.9, 46.0, 51.6, 54.0, 54.6, 71.9, 76.7, 77.06, 77.1, 77.38, 126.5, 126.9, 127.7, 130.9, 132.5, 132.9, 133.36, 133.46, 174.15, 214.32.

Example 7: Synthesis of 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor PGE₂ isopropyl ester (compound 7)

The synthesis of the title compound is schematically shown in Scheme 3. Bold figures refer to respective structures in Scheme 3.

Dimethyl-(2-oxo-4-(3-fluorophenylbutyl) phosphonate

To a stirred suspension of sodium hydride (4.17 g, 138 mmol) previously washed with n-pentane, in dry THF (250 ml) at room temperature was added dropwise a solution of dimethyl-2-oxo-propylphosphonate (23.12 g, 132.3 mmol) in THF (110 ml). The reaction mixture was stirred for 2 h, then cooled in an ice bath and treated with a solution of n-BuLi (10.2 g, 158.7 mmol) in hexane, causing a dark brown solution to be formed. Stirring was continued for 2 h at 0 °C, followed by dropwise addition of 3-fluorobenzyl bromide (25 g, 132.3 mmol) in THF (50 ml). The reaction mixture was gradually warmed to room temperature and after 3 h (TLC monitoring), it was quenched with 10% HCl (20 ml). The mixture was poured into ice-water (200 ml), extracted with CHCl₃ (2x150 ml), the organic layers were collected, washed with brine (150 ml), chromatographed on silica gel using CH₂Cl₂ and EtOAc successively as eluent, furnishing 19.5 g of a slightly yellow oil. *R*_f = 0.37 (silica gel, EtOAc:acetone 1:1)

(1S,5R,6R,7R)-6-Formyl-7-(4-phenyl benzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one

19

To a solution of the alcohol **18** (19.0 g, 53.9 mmol) in DME (100 ml), cooled to 18 °C, was added dicyclohexylcarbodiimide (DCC) (33.3, 161.8 mmol), DMSO (38.2 ml) and phosphoric acid (1.43 ml, 21.28 mmol). The temperature of the reaction mixture was kept below 25 °C for 30 min. The reaction mixture was stirred at room temperature for additional 2 hours (TLC monitoring), and the precipitate was removed

by filtration and washed with ether (2x50 ml). The combined organic layer was washed with water (50 ml) and brine (2x50 ml), the aqueous solution was extracted with ether (100 ml), the organic layers were collected and dried over sodium sulfate, filtered, and used directly for the next step. TLC R_f = 0.37 (silica gel, EtOAc:toluene 2:1).

(1S,5R,6R,7R)-6-{3-Oxo-5-(3-fluorophenyl)-1-E-pentenyl}-7-(4-phenyl benzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (20)

To a stirred suspension of NaH (1.9 g, 65.1 mmol), prewashed with n-pentane, in DME (130 ml) under nitrogen, was added dropwise dimethyl-2-oxo-4-(3-fluorophenyl) butylphosphonate (Wadsworth, Jr., W. S., et al. 1961) (19.3 g, 70.5 mmol), in DME (100 ml) and stirred vigorously for 1 h at room temperature. The mixture was then cooled to -10 °C and a solution of the crude aldehyde **19** was added dropwise. After 30 min. at 0 °C and 2 h at room temperature (TLC monitoring), the reaction mixture was neutralized with acetic acid, the solvent was removed in vacuo and the residue was dissolved in EtOAc (200 ml), and washed with water (50 ml) and brine (50 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated in vacuo. The residue was stirred with ether (100 ml), the resulting white precipitate was filtered and washed with cold ether, giving a white crystalline substance yield (17 g, 58.5%) R_f = 0.56 (silica gel, ethyl acetate : toluene 2:1)

(1S,5R,6R,7R)-6-(3S-3-Hydroxy-5-(3-fluorophenyl)-1-pentenyl)-7-(4-phenyl benzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (21)

To a stirred solution of the enone **20** (17.1 g, 34.3 mmol) in THF (20 ml) and cerium chloride ($\text{CeCl}_3 \cdot 7 \text{H}_2\text{O}$) (3.8g, 10.3 mmol) in THF:ether 1:2 (60 ml) cooled to -20 °C under nitrogen was added sodium borohydride (0.8 g, 20.57 mmol) in small portions. The reaction mixture was stirred for 2 h (TLC monitoring). The temperature was raised to ± 0 °C, then quenched by adding water (20 ml) and an aqueous solution of 10% HCl to pH 4 and extracted with EtOAc (50 ml). The organic layer was separated and washed with brine, dried on anhydrous sodium sulfate, concentrated in vacuo and

chromatographed twice on silica gel using toluene:EtOAc 2:1 and 1:1 successively as eluent, furnishing **4** (5 g) as a white crystalline product $R_f = 0.32$ (silica gel, EtOAc:toluene 2:1).

(1S,5R,6R,7R)-6-{3R-3-Hydroxy-5-(3-fluorophenyl)-1-pentyl}-7-(4-phenylbenzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (22)

To a suspension of 10% Pd/C (0.1 g) in sodium nitrite (3.6 ml, 1.8 mmol) and ethanol (15 ml) was added a solution of **21** (3 g, 6.0 mmol) in ethanol (6.0 ml). The mixture was stirred under hydrogen atmosphere for 6 h (TLC monitoring), and quenched with 1M solution of HCl. The catalyst was removed by filtration through a celite pad, washed with ethanol abs. (15 ml). The solvent was removed in vacuo. The resulting oil was dissolved in EtOAc (100 ml), and washed with brine 15% (30 ml). The water phase was washed with EtOAc (40 ml). The combined organic extracts were dried over sodium sulfate and filtered. The solvent was removed in vacuo. The residue was chromatographed on silica gel using EtOAc as eluent, which gave **5** (2.94 g), $R_f = 0.25$ (silica gel, EtOAc).

(1S,5R,6R,7R)-6-{3R-3-Hydroxy-5-(3-fluorophenyl)-1-pentyl}-7-R-hydroxy-2-oxabicyclo[3.3.0]octane-3-one (23)

To a solution of the lactone **22** (2.8 g, 5.65 mmol) in methanol (15 ml) was added potassium carbonate (0.47 g, 3.3 mmol) and the mixture was stirred at ambient temperature for 6 h (TLC monitoring). The mixture was neutralised with 10% aqueous solution of HCl and extracted with EtOAc (2x30 ml). The organic phase was dried on anhydrous sodium sulfate and evaporated to dryness. The crude product was chromatographed on silica gel using EtOAc:acetone 1:1 as eluent. The title compound **23** was obtained as a white crystalline product; yield 1.6 g, $R_f = 0.17$ (silica gel, EtOAc); ^1H NMR (CDCl_3) δ 1.2-1.4 (m, 1H), 1.54 (m, 3H), 1.8 (m, 3H), 2.1 (m, 1H), 2.2 (m, 1H), 2.3 (m, 1H), 2.6 (m, 2H), 2.67 (m, 1H), 2.8 (m, 2H), 3.60 (m, $\text{CH}_2\text{CHOHCH}_2$), 4.0 (m, CHOH) 4.92 (m, CHOC=O), 6.8-7.0 (m, 3H), 7.28 (m, 1H).

(1S,5R,6R,7R)-6-{3R-3-t-butyl dimethylsilyloxy-5-(3-fluorophenyl)-1-pentyl}-7-R-t-butyl dimethylsilyloxy-2-oxabicyclo[3.3.0]octane-3-one (24)

t-Butyldimethylsilyl chloride (2.3 g, 14.9 mmol) was added in one portion to a solution of the diol **23**, triethyl amine (2.1 ml, 14.8 mmol) and 4-dimethylamino pyridine (0.06 g, 0.1 mmol) in dichloromethane (20 ml) with vigorous stirring at room temperature for 24 h, and the reaction mixture was concentrated in vacuo. The crude product was dissolved in ethyl acetate (50 ml), washed with water (20 ml) and 5% aqueous solution of sodium hydrogen carbonate (20 ml). The organic phase was dried on sodium sulfate, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using dichloromethane as eluent to give 3 g of the product as oil. R_f = 0.68 (silica gel, ether)

(1S,5R,6R,7R)-6-{3R-3-t-butyl dimethylsilyloxy-5-(3-fluorophenyl)-1-pentyl}-7-R-t-butyl dimethylsilyloxy-2-oxabicyclo[3.3.0]octane-3-ol (25)

A solution of diisobutylaluminium hydride (DIBAL) (1.1 g, 7.43 mmol) in dry toluene (5.3 ml) was added dropwise to a stirred solution of the lactone **24** (2.7 g, 4.95 mmol) in dry THF (30 ml) at -72/-80 °C. After 1 h (TLC monitoring), the reaction mixture was quenched with methanol (5 ml) and was warmed to room temperature, and added water (50 ml), 10% aqueous solution of HCl (50 ml), extracted with EtOAc (2x50 ml). The organic layer was dried with sodium sulfate, filtered, the solvent was removed in vacuo, and the residue was chromatographed on silica gel using EtOAc and EtOAc:acetone 1:1, respectively, as eluent, to give a yellow oil product (2.7 g), R_f = 0.85 (silica gel, ethyl acetate 1:1).

13,14-Dihydro-11,15-di-t-butyldimethyl silyloxy-17-(3-fluorophenyl)-18,19,20-trinor-PGE_{2α} (26)

To a stirred suspension of 4-carboxybutyl triphenyl phosphonium bromide (8.78 g, 19.82 mmol) in THF (50 ml) under nitrogen at 0-5 °C was added potassium t-butoxide (3.89 g, 34.6 mmol), and the mixture stirred for 30 min. at room temperature. To the resultant red orange solution of ylide at -15/-10 °C was added the lactol **25** (2.7 g, 4.95 mmol) in THF (10 ml), and the mixture was stirred for 3-4 h (TLC monitoring). The reaction mixture was diluted with water (30 ml) and washed with ether (4x40 ml). The water layer was acidified with 5% aqueous solution of citric acid to pH 4 and extracted with EtOAc (2x50 ml). The organic phase was washed

with brine (30 ml), dried on sodium sulfate, and filtered. The solvent was removed in vacuo, and the slurry **26** was used directly without isolation for the next step.

13,14-Dihydro-11,15-di-t-butyl dimethyl silyloxy 17-(3-fluorophenyl)-18,19,20-trinor PGF_{2α} isopropyl ester (27)

DBU (5.28 g, 34.7 mmol) was added dropwise to a stirred solution of the crude product **26** (3.16 g, 4.96 mmol) in acetone (20 ml) at 0 °C. The mixture was allowed to warm to room temperature, and isopropyl iodide (5.05 g, 29.7 mmol) was added dropwise. After 4 h (TLC monitoring), the mixture was diluted with EtOAc (100 ml), washed with brine (30 ml), citric acid 3% (2x25 ml) and sodium hydrogen carbonate 5% (2x25 ml) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel using ether:petroleum ether 1:2 as eluent. This afforded a colourless oil, yield 1.7 g, R_f = 0.43 (silica gel, ether : petroleum ether 1:2) ¹H NMR (CDCl₃) δ 0.1 (m, 9H), 0.9 (m, 16H), 1.2 (m, 9H), 1.6-1.8 (mm, 10H), 2.12 (m, 2H), 2.22-2.33 (m, 2H), 2.6-2.9 (dm, 2H), 3.65 (m, CH₂CHOHCH₂), 3.94 (m, CH₂CHOH), 4.16 (m, CH₂CHOH), 5.0 (sept. 1H), 5.38 (m, db), 5.47 (m, db), 6.8-7.0 (dm, Ar, 3H), 7.2 (m, Ar, 1H).

13,14-Dihydro-11,15-di-t-butyl dimethyl silyloxy 17-(3-fluorophenyl)-18,19,20-trinor PGE₂ isopropyl ester (28)

Pyridinium dichlorochromate (2.43 g, 11.25 mmol) on aluminum oxide (20 g) was added in small portions to a solution of **27** (1.7 g, 2.5 mmol) in dichloromethane (30 ml) and the mixture was stirred at room temperature (TLC monitoring), filtered, and the precipitate was washed with ether: ethyl acetate 2:1. The solvent was removed in vacuo. The residue was diluted with ether (100 ml) and washed with water (30 ml), 5% aqueous solution of NaHCO₃ (3x20 ml), the organic phase was separated and dried over sodium sulfate, and evaporated in vacuo to give **28** (1.3 g), as an oil. R_f = 0.72 (silica gel, ethyl acetate).

13,14-Dihydro-17-(3-fluorophenyl)-18,19,20-trinor PGE₂ isopropyl ester (7)

Hydrogen fluoride 15% (12 ml) was added to a solution of 28 (314 mg) in acetonitrile. The mixture was stirred at room temperature for 4 h (TLC monitoring). The reaction mixture was diluted with ethyl acetate (100 ml) and washed with water (3x20 ml), dried and evaporated in vacuo. The residue was chromatographed on silica gel using ethyl acetate as eluent, which gave 7 (64 mg) as an oil, $R_f = 0.43$ (silica gel, ethyl acetate). ^1H NMR (CDCl_3) δ 1.2 (d, 6H), 1.6-1.8 (m, 6H), 1.8 (m, 2H), 2.12 (m, 2H), 2.2-2.3 (m, 2H), 2.6-2.8 (dm, 2H), 3.6 (m, $\text{CH}_2\text{CHOHCH}_2$), 4.16 (m, CH_2CHOH), 5.0 (sept. 1H), 5.38 (m, db), 5.47 (m, db), 6.8-7.0 (dm, Ar, 3H), 7.2 (m, Ar, 1H).

Pharmacology

Intraocular pressure reducing effect of he test compounds in cats and monkeys.

The compounds were tested for intraocular pressure reducing effect in animal models. The intraocular pressure was measured with a calibrated pneumotonometer. European domestic cats and cynomolgus monkeys were used as experimental animals. The cornea was anaesthetized with oxibuprocain before the measurement. The reductions in intraocular pressure after topical treatment with the PGF_{2 β} isopropyl ester (2), 17-phenyl-18,19,20-trinor-PGE₂-isopropyl ester (4), 15RS-16,16-trimethylene-methyl ester (6) and 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE₂-isopropyl ester (7) are demonstrated in Tables IV and V.

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Table IV. Intraocular pressure reducing effect of 1-10 µg of the test compounds, with effect on the EP₁ prostanoid receptor, in cats. The control eye received the vehicle only. (n= 5-6; Mean±SEM).

Prostaglandin/ Eye	Baseline pressure (mmHg)	Pressure 3 h after treatment (mmHg)
<u>2</u>		
Experimental eye	24.2 ± 2.3	15.1 ± 2.8 *
Control eye	24.5 ± 2.7	22.5 ± 3.4
<u>4</u>		
Experimental eye	22.0 ± 1.7	14.2 ± 1.7 *
Control eye	21.5 ± 1.7	18.7 ± 1.9
<u>6</u>		
Experimental eye	19.2 ± 1.7	9.5 ± 0.5 *
Control eye	19.3 ± 1.7	17.0 ± 1.3
<u>7</u>		
Experimental eye	20.4 ± -2.0	14.2 ± 0.9 *
Control eye	20.6 ± -1.8	18.4 ± 1.5

* p<0.01 (matched pair t-test between eyes)

Table V. Intraocular pressure reducing effect of the test compounds, with effect on the EP₁ receptor, in monkeys. The dose of PGF_{2 β} -isopropyl ester was 30 μ g, while that of 17-phenyl-18,19,20-trinor-PGE₂-isopropyl ester, and 15RS-16,16-trimethylene-PGE₂-isopropyl ester was 3 μ g. The control eye received the vehicle only (n=6; Mean \pm SEM).

Prostaglandin/ Eye	Baseline pressure (mmHg)	Pressure 4 h after treatment (mmHg)
<u>2</u>		
Experimental eye	17.8 \pm 1.4	14.1 \pm 1.8 *
Control eye	16.9 \pm 1.2	17.5 \pm 2.0
<u>4</u>		
Experimental eye	14.1 \pm 1.1	9.9 \pm 0.9 *
Control eye	13.9 \pm 1.0	11.5 \pm 0.8
<u>6</u>		
Experimental eye	20.9 \pm 1.6	15.3 \pm 2.4 *
Control eye	21.3 \pm 1.5	19.0 \pm 1.5

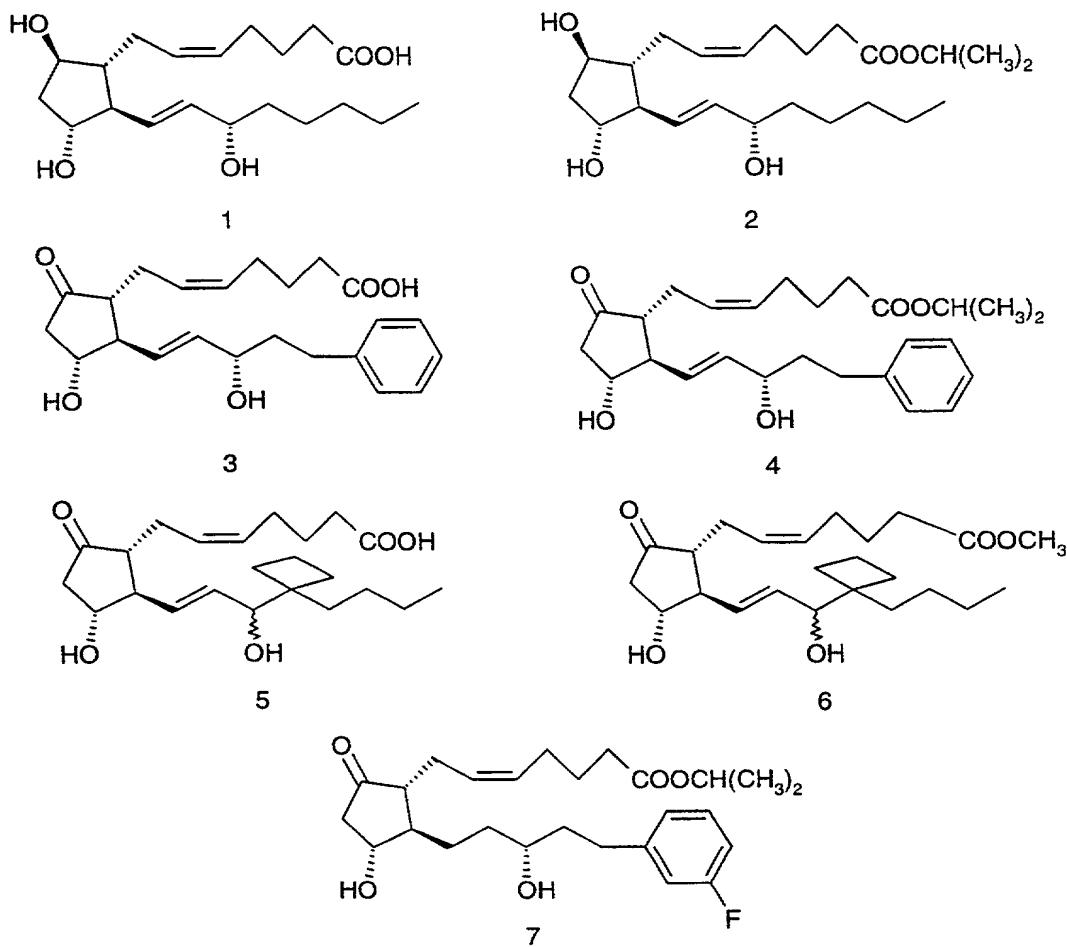
* p<0.05 (match paired t-test between the eyes)

It can be seen that both in cats and monkeys all the prostaglandin analogues with preference for the EP₁ receptor significantly reduced the intraocular pressure.

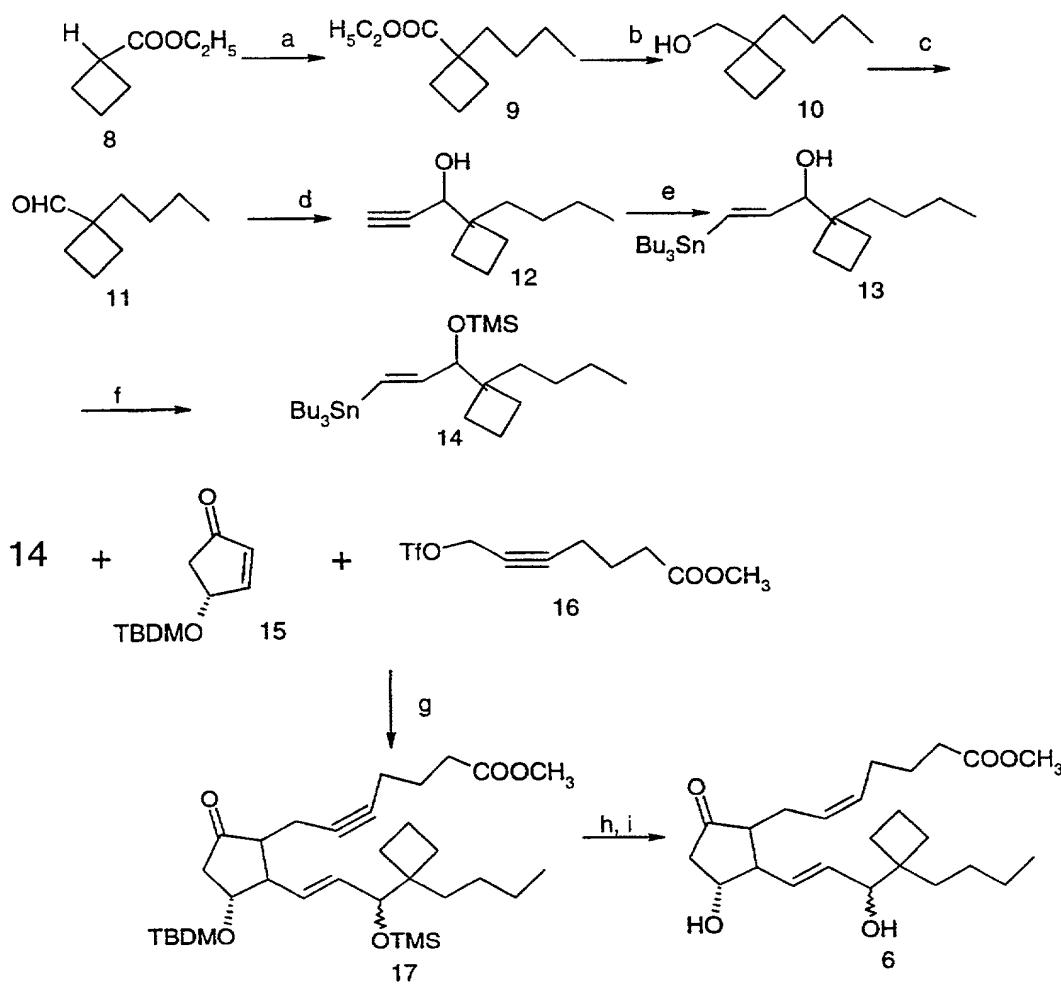
Accordingly, the present invention discloses that compounds with selective stimulatory effect on EP₁ receptors reduce the intraocular pressure, and that such compounds cannot have any melanogenic effect, or at least have significantly reduced effect in the eye since the pigment producing cells, the melanocytes, lack the EP₁

receptor in man. Thus, the common side-effect of increased iridial pigmentation can be avoided during chronic therapy with prostaglandins selective for EP₁ receptors.

Scheme 1



Scheme 2



Reagents

a. *N*-isopropylcyclohexyl amine / THF, *n*-BuLi, ethylcyclobutanecarboxylate/ DMSO

b. DIBAL-H, / toluene

c. DCC/ DME, DMSO, *H*₃PO₄,

d. Lithium-acetylide-ethylene diamine, DMSO

e. tributyltin hydride, AIBN

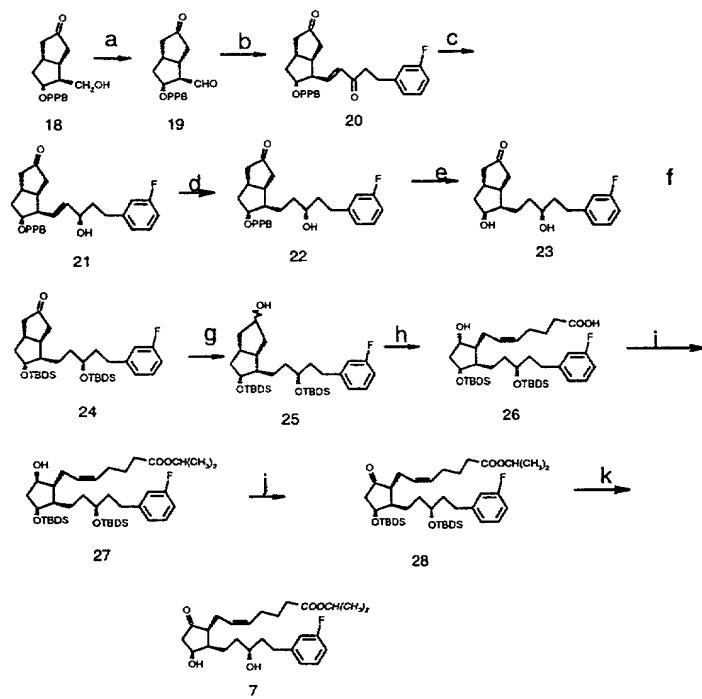
f. Trimethylsilyl chloride (TMSCl), imidazole/ DMF

g. Li₂CuCN(CH₃)₂, TMSCl, triethylamine, 4-*t*-butyl-dimethylsilyloxy-2-cyclopentenone, 1-tributyltin-4,4,-trimethylene-3-trimethylsilyloxy-1-octene, methyl-2-yn-8-octanoate

h. Pd-BaSO₄, quinoline,

i. HF/THF

Scheme 3



Reagents

- a. DCC, DMSO, H₂SO₄, DME, H₃PO₄
- b. NaH, dimethyl-2-oxo-4-(3-fluorophenyl)-butylphosphonate
- c. NaBH₄, CeCl₃.7H₂O/THF
- d. Pd/C, NaNO₂/ THF
- e. K₂CO₃/Methanol
- f. TBDS, TEA, 4-dimethylamino pyridine/dichloromethane
- g. DIBAL-H/ THF
- h. 4-carboxybutyl triphenyl phosphonium bromide
potassium t-butoxide, THF
- i. DBU, isopropyl iodide/ acetone
- j. pyridinium chlorochromate, aluminium oxide/ dichloromethane
- k. HF/acetonitrile

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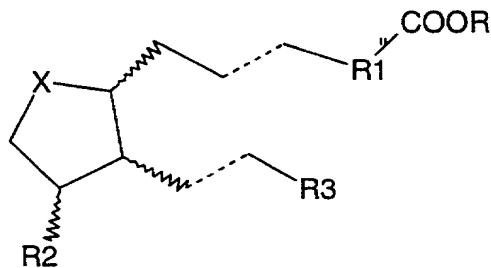
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CLAIMS

1. A composition for the treatment of glaucoma and ocular hypertension comprising a therapeutically active and physiologically acceptable amount of a prostaglandin analogue which is a selective agonist for EP₁ prostanoid receptors, or a pharmaceutically acceptable salt or ester thereof.
2. The composition according to claim 1, wherein the prostaglandin analogue is derived from PGF or PGE type prostaglandins.
3. The composition according to claim 1 or 2, wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably C₁₋₁₀ alkyl, cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl, arylalkyl, preferably aryl-C₂₋₅ alkyl, or heteroaryl;

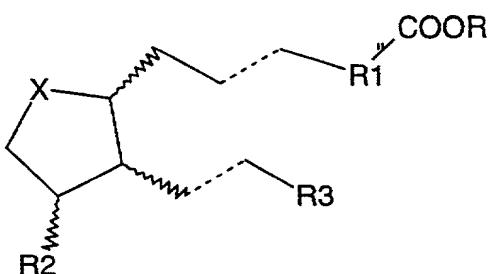
R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by a heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably C₃₋₇ cycloalkyl, cycloalkenyl, preferably C₃₋₇ cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, preferably C₁₋₁₀ alkyl, especially C₁₋₆ alkyl, or a cycloalkyl, preferably C₃₋₈ cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C₁₋₅ alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

4. The composition according to claim 1, 2 or 3, wherein the prostaglandin analogue is 15(R,S)-16,16-trimethylene-PGE₂ or an alkyl ester thereof.
5. The composition according to claim 1, 2 or 3 wherein the prostaglandin analogue is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE₂ or an alkyl ester thereof.
6. A method of treating glaucoma or ocular hypertension in a subject's eye, which method comprises contacting the surface of the eye with an effective intraocular pressure reducing amount of a therapeutically active and physiologically acceptable prostaglandin analogue which is a selective agonist for EP₁ prostanoid receptors, or a pharmaceutically acceptable salt or ester thereof.
7. The method according to claim 6, wherein the prostaglandin analogue is derived from PGF or PGE prostaglandins.
8. The method according to claim 6 or 7, wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably C_{1-10} alkyl, cycloalkyl, preferably C_{3-8} cycloalkyl, aryl, arylalkyl, preferably aryl- C_{2-5} alkyl, or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by a heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably C_{3-7} cycloalkyl, cycloalkenyl, preferably C_{3-7} cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, preferably C_{1-10} alkyl, especially C_{1-6} alkyl, or a cycloalkyl, preferably C_{3-8} cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C_{1-5} alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably C_{3-8} cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with C_{1-3} alkyl, C_{1-3} alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

9. The composition according to claim 6, 7 or 8, wherein the prostaglandin analogue is 15(R,S)-16,16-trimethylene-PGE₂ or an alkyl ester thereof.

10. The composition according to claim 6, 7 or 8 wherein the prostaglandin analogue is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE₂ or an alkyl ester thereof.
11. The method according to any one of claims 6-10, wherein a therapeutically active and physiologically acceptable composition containing said prostaglandin analogue is administered topically on the eye 1-3 times daily.
12. Use of a prostaglandin analogue which is a selective agonist for EP₁ prostanoid receptors as defined in any one of claims 1 to 4 for the preparation of a medicament for treatment of glaucoma and ocular hypertension.

DECLARATION
and
POWER OF ATTORNEY

U.S. NATIONAL PHASE OF INTERNATIONAL APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **Prostaglandin Derivatives Devoid of Side-Effects for the Treatment of Glaucoma**, the specification of which was filed as International Application No. PCT/SE98/01368 on July 10, 1998,

[] and was amended under Article 19 on _____.
 (if applicable)

[] and was amended under Article 34 on _____.
 (if applicable)

[x] and was assigned U.S. Application Serial No. 09/445,919
 and was amended on _____.
 (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits and/or U.S. Provisional application priority benefits under Title 35, United States Code, §119 of any foreign application(s) or U.S. Provisional applications for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign and U.S. Provisional Application(s)			Priority Claimed	
Number	Country	Day/Month/Year Filed	Yes	No
9702706-4	Sweden	11 July 1997	X	

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this

application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulation, §1.56(a) which occurred between the filing date of the prior application and the PCT international filing date of this application:

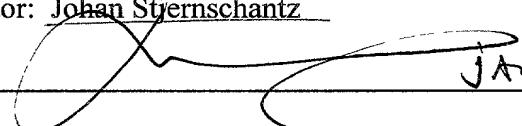
(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

I hereby appoint Holly D. Kozlowski, Registration No. 30,468; Ronald J. Snyder, Registration No. 31,062; James D. Liles, Registration No. 28,320; Lynda E. Roesch, Registration No. 29,696; Phillip A. Rotman II, Registration No. 38,290; Martin J. Miller, Registration No. 35,953; Victor C. Moreno, Registration No. 40,732; Jackie A. Zurcher, Registration No. 42,251; John V. Harmeyer, Registration No. 41,815; Scott N. Barker, Registration No. 42,292; and Geoffrey L. Oberhaus, Registration No. 42,955, c/o Dinsmore & Shohl, 1900 Chemed Center, 255 East Fifth Street, Cincinnati, Ohio 45202 (513) 977-8200, my attorneys, with full power in each of them, of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

The undersigned hereby authorizes the above-named U.S. attorneys to accept and follow instructions from **Pharmacia & Upjohn AB** as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the undersigned and the aforementioned U.S. attorneys. In the event of a change in the firm or persons from whom instructions may be taken, the aforementioned U.S. attorneys will be so notified in writing by the undersigned.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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